

REMARKS

Status of the Claims

The pending office action addresses claims 1, 2, 5, 6, 8-17, 19-23, 26-30, 32-35, 37-41, and 43-45 (the remainder of claims 1 to 57 being withdrawn as a result of a restriction requirement), with claims 1, 19, and 37 being independent. All claims stand rejected. By this response, Applicants have canceled claims 2-7, 18, 24-25, 31, 36, 42, and 46 to 57, added new claims 58 to 64, and amended claims 1, 8-15, 17, 19 and 28. Upon entry of this amendment, claims 1, 8-17, 19-23, 26-30, 32-35, 37-41, 43-45, and 58 to 64 will remain pending in the application with claims 1, 19, 37, and 58. being independent. Applicant respectfully requests reconsideration.

Claim Rejections - 35 USC §112, first paragraph

Written Description

The Examiner states that claims 1, 2, 5, 6, 8-17, 19-23, 26-30, 32-35, 37-41 and 43-45 are rejected under 35 U.S.C. 112, first paragraph as failing to comply with the written description requirement. Specifically, the Examiner states that:

The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Specifically, the claims recite a method of preserving a biomaterial having a membrane and a transporter molecule or transporter protein in which the biomaterial is exposed to a preservation agent. Some of the instant claims recite the invention more narrowly, as a mammalian cell instead of a biomaterial, or in which the preservation agent is a non-metabolizable carbohydrate. But, even the claims that "recite a method of preserving a mammalian cell also recite that the genus of any transporter protein may be used in the method. Thus, the claims recite three vast genera for each of which only a very few species are disclosed. The genus of a biomaterial having a membrane reads on things as different as whole animals, whole plants, packaged foods, medical supplies, packaged cosmetics, pharmaceuticals and nutraceuticals, etc. But, the specification makes it clear that the biomaterial having a membrane is a mammalian cell.

The genus of a transporter molecule includes any type of molecule as well as all proteins- e.g., lipids, polysaccharides, polynucleotides, organic molecules. In the specification, however, the only transporter molecules or transporter proteins disclosed are the trans-membrane GLUT transporter proteins for monosaccharides and disaccharides. The genus of a preservation agent reads on any preservative, e.g., an antibiotic, insecticide, antioxidant, nitrite, sulphite, EDTA or formaldehyde. The only preservative agents disclosed in the specification are glucose and several non-metabolizable glucose analogues, sucrose; mannose, galactose and a hexose. A sufficient written description of a genus of cells, proteins or preservation agents (monosaccharides or disaccharides) may be achieved by a recitation of structural features common to each member (species) of the genus, **which features constitute a substantial portion of each member of the genus.** The only recited structural feature of the genus of biomaterial in these claims (i.e., any biocompatible material having any kind of membrane) does not constitute a substantial portion of each species in the genus, as the remainder of the structure is completely undefined and the specification does not define the remaining structural features necessary for members of the genus to be selected. The structural features needed for the transporter molecule or protein to function in transporting the monosaccharide or disaccharide across a cell membrane also are not recited. As noted above, the specification makes it clear that, for a preservation agent to function in the claimed method it must be structurally a mono- or disaccharide. Therefore, one skilled in the art cannot reasonably conclude that Applicants had possession of the claimed invention at the time the instant application was filed.

Consequently, there is no evidence that a sufficient number of representative species of these very large genera were in the possession of the inventors at the time of filing. To satisfy the written description aspect of 35 U.S.C. 112, first paragraph, for a claimed genus of molecules, it must be clear that: (1) the identifying characteristics of the claimed molecules have been disclosed, e.g., structure, physical and/or chemical characteristics, functional characteristics when coupled with a known or disclosed correlation between function and structure, or a combination of these; and (2) a representative number of species within the genus must be disclosed. Because only a very limited number of species of each of the claimed genera are disclosed, the claims fail to satisfy the written description requirement.

According to the Examiner, three broad genera cause written description problems with the claims. These broad genera are said to be (1) biomaterial; (2) transporter molecule; and (3) preservation agent. In particular, the Examiner asserts that the application lacks sufficient disclosure of identifying characteristics and representative species for these genera. Applicant disagrees.

First, the Examiner does not properly state the requirements of a sufficient written description. The written description need not provide identifying characteristics *and* a sufficient number of representative examples as suggested by the Examiner, but only sufficient identifying characteristics *or* a sufficient number of representative examples. MPEP § 2163 (II)(A)(3)(ii) at 2000-182. Further, under certain circumstances, disclosure of *a single species may be enough* to support a genus. See, e.g., *Rasmussen*, 650 F.2d 1212, 1214, 211 USPQ 323, 326-27 (CCPA 1981)(disclosure of a single method of adheringly applying one layer to another was sufficient to support a generic claim to 'adheringly applying' because one skilled in the art reading the specification would understand that it is unimportant how the layers are adhered, so long as they are adhered); *In re Herschler*, 591 F.2d 693, 697, 200 USPQ 711, 714 (CCPA 1979) (disclosure of corticosteroid in DMSO sufficient to support claims drawn to a method of using a mixture of a 'physiologically active steroid' and DMSO because 'use of known chemical compounds in a manner auxiliary to the invention must have a corresponding written description only so specific as to lead one having ordinary skill in the art to that class of compounds. Occasionally, a functional recitation of those known compounds in the specification may be sufficient as that description.');

In re Smythe, 480 F.2d 1376, 1383, 178 USPQ 279, 285 (CCPA 1973) (the phrase 'air or other gas which is inert to the liquid' was sufficient to support a claim to 'inert fluid media' because the description of the properties and functions of the air or other gas segmentizing medium would suggest to a person skilled in the art that appellant's invention includes the use of 'inert fluid' broadly.). MPEP § 2163 (II)(A)(3)(ii) at 2000-183.

In addition, the Examiner bears a heavy burden where, as is the case here, *original claims* are rejected for lacking written description as "[t]here is a strong presumption that an adequate written description of the claimed invention is present when the application is filed. *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976) ('we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would

not recognize in the disclosure a description of the invention defined by the claims’).” MPEP § 2163 (I)(A) at 2000-174.

Further with respect to the law, when analyzing written description issues, “[t]he entire claim must be considered.” MPEP § 2163 (II)(A)(1) at 2000-176. Finally, “[t]he absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. 112, para. 1, for lack of adequate written description.” MPEP § 2163 (II)(A)(1) at 2000-177.

In this case, the Examiner takes words or terms out of context from the claims (i.e., does not consider the claim as a whole), and then requires that the applicants recite structural features of these words or terms in the claims, when in fact the words in the claims are common terms well understood in the art.

First, taking the “biomaterials” genus, the Examiner asserts that the recited biomaterial would read on medical supplies or packaged cosmetics. No such interpretation could be possible when reading either the specification or the claims. Turning to the claims, “biomaterial” is not recited in a vacuum – the biomaterial must have a membrane and a transporter molecule that is effective to transport preservation agents across the membrane. Certainly medical supplies and packaged cosmetics do not have membranes with a transporter molecule to transport preservation agents across the membrane. In addition, to the extent there is any ambiguity as to the scope of this genus, the specification provides clarity:

[0014] The biomaterial can be any cell or organism that has at least one transporter, e.g., a mammalian cell with a glucose transporter. The biomaterial can be selected from the group consisting of organs, tissues, isolated primary cells, stem cells, cell-lines, bone marrow, embryos, platelets, lymphocytes, hepatocytes, osteoblasts, spermatozoa, granulocytes, red blood cells, dendritic cells, oocytes, and plant cells. The invention is particularly useful for preservation of nucleated cells, as these cells often react poorly to conventional preservation protocols.

Regardless, Applicant has amended the claims so that each recites “cells,” “mammalian cells,” or “nucleated mammalian cells” as the biomaterial. The meaning of “cells” is sufficiently well established in the art that no further identifying structure of the cells should need to be recited in

order meet the written description requirement. Despite this, each claim further requires that the cell have a membrane and a transporter molecule that can transport a preservation agent across the membrane. This class of materials is well defined.

In addition to the clear definition of the biomaterial used in the application, a number of examples are provided, both for the biomaterial and for the biomaterial as used in the claimed methods in their entirety. The Examiner admits that the use of the methods on mammalian cells is both described and enabled, but the specification provides description of much more than just mammalian cells. For example, the specification characterizes the transport of sucrose across cell membranes in plants:

[0050] In another embodiment, the transporter proteins is a sucrose transporter protein. The regulation of sucrose transport in plants has a major impact on plant growth and productivity. Through photosynthesis, plants fix atmospheric carbon dioxide into triose phosphates, which are then used to produce sucrose and other carbohydrates. These carbohydrates are then transported throughout the plant for use as energy sources, carbon skeletons for biosynthesis and storage for future growth needs. Sucrose is the major form of transported carbohydrate. Sucrose is loaded into the phloem by a proton/sucrose symporter located in the phloem plasma membrane and then distributed throughout the plant. The ability of plant cells actively to transport sucrose across the plasma membrane so that the sucrose that is mobilized in the phloem can be taken into cells for use is a critical step in sucrose utilization (Riesmeier et al. (1993) Plant Cell. 5:1591-1598; Hirose, et al. (1997) Plant Cell Physiol. 38:1389-1396).

[0051] Various transporter proteins responsible for transporting substances through membranes have already been identified in plants, and in some cases DNA sequences which code for such transporter proteins are available. cDNA sequences which code for plant sucrose transporters have been described, for example for potatoes (p 62 and StSUT1) and spinach (S21 and SoSUT1) (WO 94/00574; Riesmeier et al., (1993) Plant Cell 5:1591-1598; Riesmeier et al., (1992) EMBO J. 11: 4705-4713), for Arabidopsis thaliana (suc1 and suc2 genes; EMBL gene bank: Access No. X75365), Plantago major (EMBL gene bank: Access No. X75764), L. esculentum (EMBL gene bank: Access No. X82275) and Nicotiana tabacum (EMBL gene bank: Access Nos. X82276 and X82277). In the case of the sucrose transporters, it was possible to clone cDNA sequences coding for these transporters from spinach and potato by developing an

artificial complementation system in *Saccharomyces cerevisiae* (Riesmeier et al. (1992) EMBO J. 11: 4705-4713; Riesmeier et al., (1993) Plant Cell 5: 1591-1598). It has likewise been possible to show for the sucrose transporter that a reduction in the activity leads to a great inhibition of growth of potato plants. Furthermore, the leaves of the affected plants are damaged, and the plants produce few or no potato tubers (Riesmeier et al. (1994) EMBO J. 13: 1-7).

The specification further goes on to characterize the transport of small carbohydrate sugars that can be used for preservation in *E. coli*:

[0052] In yet another embodiment, the transporter protein is a mannose transporter protein. Many sugars are transported into *E. coli* by phosphoenolpyruvate-dependent phosphotransferase systems (PTS). Such sugars include glucose, fructose, mannose, galactitol, mannitol, sorbitol, xylitol and N-acetylglucosamine. They are phosphorylated as they are transported into the cell. For example, glucose enters the cell as glucose-6-phosphate. The phosphate group is transferred from phosphoenol pyruvate (PEP) through a series of intermediary proteins some of which are common to all PTS sugar transport systems and some of which are specific for an individual PTS sugar transport system. The former include EI and HPr; the latter is the EII complex which has several functional domains that may or may not exist as separate or distinct entities.

The descriptions in the specification apply far more broadly than just mammalian cells. Indeed, mammalian cells, and in particular the hepatocytes that appear in the examples, are the most difficult case for preservation. With the most difficult cases exemplified, and the further specific description for use in other types of cells, and the category of biomaterials recited in the claims in terms whose meaning is well established in the art – these claims and this specification more than meet the written description requirement.

The second of the genera identified by the examiner is “transporter molecules.” The Examiner posits that transporter molecule includes “any type of molecule as well as all proteins.” Again, the term “transporter molecule” is not recited in a vacuum. The recited transporter molecule is included in a cell having a membrane and performs the function of transporting a preservation agent (in some claims variously defined as small carbohydrate sugars, non-metabolizable sugars, and specific groups of sugars) across the membrane. In this context,

transporter molecules are well defined in the art as shown by the large number of publications cited in the specification that characterize them (see also the entry for “membrane transport proteins” at wikipedia.org which provides clear characterizations).

The Examiner admits that the GLUT family of transporters is described and enabled in the application, but, again, there are more examples in the specification than just the 13 GLUT family members that are described in detail. In general, the specification provides:

[0015] The transporter can be a selected from the group consisting of a glucose transporter (GLUT), a sucrose transporter, a mannose transporter, a galactose transporter, and a hexose transporter, or any combination thereof. In a preferred embodiment, the transporter is a glucose transporter (GLUT), which exist on all mammalian cells.

The specification goes on to provide, as already quoted extensively above, very specific descriptions of sucrose transporters and mannose transporters. In addition, in virtually all of the claims, the family of transporter molecules is limited by a family of preservation agents (such as sugars or specific sugars) that the transporter molecule must transport. That the transporter molecules must transport specific compounds across a cell membrane significantly limits the scope of “transporter molecule” in the claims.

Given the understanding in the art, and the number of examples in the specification, as well as the context provided by the claims themselves when considered as a whole (as they must be), the claims presented clearly meet the written description requirement.

The third of the three genera identified by the examiner is the “preservation agent.” Again, the Examiner takes this term out of context and defines it very broadly. Looking to claim 1 as presently presented for example, the preservation agent must be something that can be transported across a cell membrane by a transporter molecule in an amount sufficient to preserve the cell that is subsequently frozen. The Examiner’s examples of antibiotics, insecticides, antioxidants, nitrites, sulphites, EDTA or formaldehyde do not meet these recitations of the claim as a whole – unless of course one of those elements is a cryopreservation agent that can be transported across a cell membrane to sufficient levels by one of the recited transporter molecules.

While the Examiner has suggested that the class of preservation agents could be limited to mono- and di-saccharides – Applicant notes that these terms do not appear in the specification, however, their synonym “sugar” does, and this term is used in a number of the claims for the preservation agent.

Applicant has also amended the claims to change their dependency so that certain of the dependent claims list specifically the cell types, transporter molecules, and preservation agents that are specifically described in the specification. See, for example, claims 16, 30 and 41. In fact, claim 41, as originally presented, is limited to the biomaterial being a nucleated mammalian cell, the transporter molecule being a GLUT, and the preservation agent being 3OMG – given the Examiner’s admission that such a claim would be described and enabled, Applicant questions why this claim was rejected.

Enablement

The Examiner states that claims 1, 2, 5, 6, 8-17, 19-23, 26-30, 32-35, 37-41 and 43-45 are rejected under 35 U.S.C. 112, first paragraph because the specification, while being enabling for the genera of: 1) a mammalian cell as the biomaterial having a membrane that is preserved by the claimed method, 2) a glucose transporter protein or GLUT protein as the transporter protein or transporter molecule, and 3) glucose, a non-metabolizable glucose analogue, a hexose, sucrose, mannose or galactose as the preservation agent, does not reasonably provide enablement for the genera in the claimed method of any biomaterial having a membrane, any transporter molecule/protein and any preservation agent. As a result, the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims. Specifically, the Examiner states that:

The factors to be considered in determining whether or not undue experimentation is required are summarized in *re Wands* 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir.1988). The court in *Wands* states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.'" (*Wands*, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be

predicated on the basis of quantity of experimentation required to make or use the invention .. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (Wands, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the breadth of the claims, (2) the nature of the invention, (3) the state of the prior art, (4) the relative skill of those in the art, (5) the predictability or unpredictability of the art, (6) the amount or direction or guidance presented, (7) the presence or absence of working examples, and (8) the quantity of experimentation necessary. Although the quantity of experimentation alone is not dispositive in a determination of whether the required experimentation is undue, this factor does play a central role. For example; a very limited quantity of experimentation may be undue in a fledgling art that is unpredictable where no guidance or working examples are provided in the specification and prior art, whereas the same amount of experimentation may not be undue when viewed in light of some guidance or a working example or the experimentation required is in a predictable established art. Conversely, a large quantity of experimentation would require a correspondingly greater quantum of guidance, predictability and skill in the art to overcome classification as undue experimentation. In Wands, the determination that undue experimentation was not required to make the claimed invention was based primarily on the nature of the art, and the probability that the required experimentation would result in successfully obtaining the claimed invention. (Wands, 8 USPQ2d 1406). Thus, a combination of factors which, when viewed together would provide an artisan of ordinary skill in the art with an expectation of successfully obtaining the claimed invention with additional experimentation would preclude the classification of that experimentation as undue. A combination of Wands factors, which provide a very low likelihood of successfully obtaining the claimed invention with additional experimentation, however, would render the additional experimentation undue.

1. Breadth of the claims.

The claims are very broad because they recite a method of preserving any biomaterial having a membrane and a transporter molecule by exposing it to a preservation agent.

2. The nature of the invention.

The invention is designed to provide a novel method for preserving a mammalian cell.

3. The state of prior art.

See the discussion of Toner et al. (US 6,127,177), Gould et al. ("The glucose transporter family: structure, function and tissue-specific expression." Biochem J 295:329-341,1993), and Pescero et al. ("Glucose metabolism by trout (*Salmo trutta*) red blood cells," J Comp Physiol B 162:448-454, 1992 below. Curtis (ys 2003/0009024 A1) was discussed in the previous Office action.

4. The relative skill in the art.

The relative skill in the art as it relates to the method of the invention is characterized. by that of a M.D. or Ph. D. level individual.

5. The level of predictability in the art.

Because the effect of exposing any biomaterial having a membrane and a transporter molecule to a preservation agent is not known, particularly because each term is so broad that it encompasses a vast set of highly different and unrelated things, the specification needs to have more detail so that the properties of each combination of these three things will be predictable and so that one of skill in the art would know which three specific, concrete things to use together so that the claimed method will work. Because the prior art and the instant specification disclose that the biomaterial is a mammalian cell, the transporter molecule is a GLUT protein and the preservation agent is glucose, a non-metabolizable glucose analogue or one of several disaccharides, it cannot be predicted that any membrane-containing biomaterial, any transporter molecule and any preservation agent would retain the structural and functional properties of the specifically disclosed species so that the claimed method will work.

6. The amount of guidance present.

As noted above, Applicants have provided guidance only for the biomaterial of a mammalian cell, the transporter molecule of a GLUT protein (the specific GLUT protein is not indicated) and the preservation agent of glucose, a non-metabolizable glucose analogue or one of several disaccharides.

7. The existence of working examples.

The limited guidance mentioned above is presented in working examples.

8. The quantity of experimentation necessary.

To prove that any biomaterial having a membrane, any transporter molecule or protein and any preservation agent may be used in the claimed method, many experiments would have to be conducted under a wide range of conditions in order to determine which species from each genus may be used together. 'In these experiments, many membrane-

containing biomaterials would have to be studied to determine which transporter molecules they contain: A preservation agent would have to be identified for each transporter molecule. The biomaterial would have to be tested under many different physical conditions (ranges of temperature, pH, dryness, oxygen content, immersion in different solutions, etc.) to determine under which conditions the transporter molecule can transport the preservation agent into the biomaterial.

These types of experiments and data are missing from the specification. A great deal of experimentation is needed to establish that any membrane-containing biomaterial, any transporter molecule and any preservation agent may be used in the claimed method, because these genera are claimed, while very few species of each genus are disclosed. Even if one combination of biomaterial, transporter molecule and preservation agent could be made and identified, by random, trial and error construction and testing, without a very large amount of data, such a result could not be expected with a different biomaterial, transporter molecule and preservation agent, particularly when tested in a different assay, or under different assay conditions, than the first combination of components. In view of the foregoing, the claims fail to satisfy the enablement requirement.

First, as with the written description rejection above, Applicant believes that at least some of the dependent claims are even more narrow than those that the Examiner believes would be enabled as stated in the rejection. See, for example, claims 16, 30, and 41.

Further, the Examiner takes the same impermissible approach to enablement that the Examiner takes with respect to written opinion – that is, taking three claim terms out of the context of the claim and asserting that they are three broad genera. The rest of the enablement rejection is based on these purportedly broad genera.

As noted extensively above, the teachings of the specification describe in great detail enabling information for a variety of cell types and not just for mammalian cells as there is significant descriptive information for applying the methods of the claims to at least plant cells and *E. Coli*. Further, the Examiner makes the most powerful case for the claims being enabled for more than just mammalian cells by citing the Pesquero reference for the obviousness of non-metabolizable glucose uptake – the cells that are applied in this reference belong to the *Salmo trutta*, or brown trout, which is not a mammal. In applying the *Wands* factors, the Examiner

includes a variety of biomaterials that could not possibly meet the recitations of the claim considered as a whole, and ignores all teaching in the application other than that for mammalian cells. As noted extensively above, the specification uses mammalian hepatocytes in many of the examples because this represents one of the most difficult preservation cases. Substantial information is provided as to how to preserve many other different kinds of cells. Under these circumstances, a person of ordinary skill can make and use the invention with those other cells.

As to the transporter molecules, again, the specification provides information for a variety of different transporters beyond the GLUT family. If a cell can uptake the preservation agent through a membrane transporter – it can be used in the method.

Turning now to the *In re Wands* factors, the Examiner has not correctly applied the first two of the *Wands* factors. The rejection specifically ignores limiting claim language as to the first factor and thus assumes very broad claims instead of the claims as actually presented, and specifically ignores the teaching as to other than mammalian cells in the second factor to define the “nature of the invention” far too narrowly for the application as written. The rejection makes these same two errors in regard to the fifth factor as well, arguing that the art is unpredictable because the claims are broad (which, in fact, they are not). Further, the specific examples in the specification represent the most difficult case. It would be more, rather than less, predictable to preserve the described potato cells using the described sucrose transporters.

These same errors are also present in the sixth and eighth factors where the rejection ignores the guidance that is not related to mammalian cells. Regarding the comment that “a great deal of experimentation is needed,” Applicant notes that the “quantity of experimentation” *Wands* factor is not merely quantitative:

"[A]n extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance." *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977). " 'The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.' " *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (citing *In re Angstadt*, 537 F.2d 489, 502-04, 190 USPQ 214, 217-19

(CCPA 1976)). Time and expense are merely factors in this consideration and are not the controlling factors. *United States v. Teletronics Inc.*, 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988), cert. denied, 490 U.S. 1046 (1989). MPEP § 2164.06 at 2100-201 (emphasis added).

. . . Time and difficulty of experiments are not determinative if they are merely routine. . . . *In re Wands*, 858 F.2d at 737, 8 USPQ2d at 1404.

Here, where the most difficult cases are provided in the examples, and substantial further guidance is provided for other examples, and the Examiner believes that it is obvious to use experiments from still other examples (i.e., the brown trout), application of the invention to other cells having membranes with transporter molecules that uptake preservation agents is merely routine – and the Examiner provides no evidence or reasoning to the contrary as the rejection does not take into account these factors.

The claims are, for at least these reasons, properly enabled.

Claim Rejections - 35 USC §112, second paragraph

The Examiner states that claim 15 is rejected under 35 U.S.C. 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner states that:

This claim recites the limitation "non-metabolizable preservation agent." There is insufficient antecedent basis for this limitation in the claim. Appropriate correction is required. The term may be amended to "preservation agent," as recited. in claim 1.

Applicant has amended this claim to correct this issue.

Claim Rejections - 35 USC §102

The Examiner has rejected claims 1, 2, 5, 6, and 8-14 under 35 U.S.C. 102(b) as being anticipated by Toner et al. (US 6,127,177), as evidenced by Gould et al. (Biochem. J. 295:329-341 (1993)). Specifically, the Examiner states:

Toner et al. disclose a method for preserving mammalian cells, such as fibroblasts, hepatocytes, bone marrow cells and granulocytes, comprising the steps of

exposing them to a solution of a preservation agent, a mono- or disaccharide, such as glucose, freezing them, and storing them in the frozen state (see cols. 1 and 2; col. 4, lines 10-55; and Fig. 1). At least a portion of these frozen cells may be recovered in a viable state (see Fig. 1; col. 5, lines 10-23 and 46-67; col. 8, lines 51-64; and cols. 12-14). The preservation agent is removed from the cells by thawing the frozen cells, incubating them in fresh cell culture medium- DMEM- which reverses the poration of the cell membrane, and washing the cells and resuspending them in additional volumes of cell culture medium, which further dilutes the preservation agent (see col. 5, lines 62-67; and col. 6, lines 47-64).

Could et al. disclose that mammalian cells naturally possess a variety of glucose transporter proteins (GLUT's). at least seven different types, and that the type of GLUT depends on the type of tissue (see pp. 329-330). Thus, in the method of Toner et al., the transporter molecule or transporter protein is part of and is present in the mammalian cells and is able to import sugars such as glucose.

In view of the foregoing, a holding of anticipation is required.

The Toner reference teaches preservation of cells using otherwise impermeable sugars such as trehalose. These otherwise impermeable sugars can be loaded intracellularly because Toner porates the cells – that is, temporary pores are created using self-assembling pores, electroporation or the like: “The present invention provides a method for preserving living cells that begins with the reversible poration of the cell membranes. . . . Once porated, the biological material is loaded to a predetermined intracellular concentration with a bio-preservation agent such as a sugar having bio-preservation properties.” Col. 1, lines 56-64. Glucose is named once in the Toner reference as a sugar that can have a preserving effect, but the reference expressly states that, “[h]owever, using the method of the invention, small carbohydrate sugars such as trehalose, sucrose and maltose, to which mammalian cell membranes are not practically permeable, may be loaded . . .” Col. 4, lines 58 to 62.

The Toner reference never actually exposes a mammalian cell to glucose, and if it did, it would come only after the cell membrane had been porated – with the sugar entering the cell through the pores (which are specifically designed to admit sugars that are larger than glucose). Claim 1, as well as the dependent claims from claim 1 that were rejected along with claim 1, recites that it is the transporter molecule that transports the preservation agent to load the cells

with the preservation agent to a desired concentration sufficient for preserving the cells. As Toner loads through pores, even if glucose were applied to a mammalian cell (which it is not), it would not include "the transporter molecule transporting the preservation agent across the membrane to load the cells with the preservation agent to a desired concentration sufficient for preserving the cells" as Toner applies pores for this purpose. Accordingly, Toner cannot anticipate claim 1 or its dependent claims.

Claim Rejections - 35 USC §103

Claims 1, 2, 5, 6, 8-17, 19-23, 26-30, 32-35, 37-41, and 43-45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Toner et al. (US 6,127,177) in view of Gould et al. (Biochem. J. 295:329-341 (1993)); and Pescero et al. (J. Comp. Physiol. B 162:448-454 (1992)). Specifically, the Examiner states:

The teachings of Toner et al. and Gould et al. are discussed above. Toner et al. do not disclose using a non-metabolizable carbohydrate as the preservation agent.

Pescero et al. disclose that 3-O-methyl-glucose (OMG) is a non-metabolizable carbohydrate and glucose analogue that is taken up by mammalian cells through their cell membranes. Glucose, a fuel source, is the most widely used monosaccharide in vertebrate cells, and vertebrates have a higher plasma concentration of glucose than of other monosaccharides. Glucose is metabolized in the glycolytic cycle to generate carbon dioxide, ATP and lactic acid (see p. 448). More acid, lactic acid, is produced from glucose than from other glycolytic intermediates, even under aerobic conditions (see p. 450, left col.). Because OMG is not metabolized, it is detectable in experiments even after 1000 min. (see p. 449, Fig. 1). Thus, there is no acid production from OMG.

It would have been obvious to one of ordinary skill in the art at the time of the invention to use a non-metabolizable glucose analogue, such as OMG, in the method of Toner et al., because Pescero et al. disclose that glucose, although readily taken up by cells, is also readily metabolized to lactic acid in the glycolytic cycle. One of ordinary skill in the art would have known that the glycolytic cycle produces other acid intermediates as well, such as pyruvic acid. Pescero et al. disclose that OMG, which is not metabolized, does not produce acid. One of ordinary skill in the art would have recognized the advantage of using a preservation agent that is stable and that does not drop the pH of the culture medium (by causing

acid production), because he would have known that the preserved cell culture would have lasted longer and that the cells would have been in better health, by being in a medium at a more desirable pH.

Regarding the concentration of the preservation agent, Toner et al. disclose that the concentration in their method is a low level, less than or equal to 1. M (see col. 1, lines 66-67). Toner et al. also disclose concentrations of 0.2 M and 0.4 M (see col. 9, lines 47-56; and col. 11, lines 28-43). Thus, it would have been obvious one of ordinary skill in the art at the time of the invention to use 0.2-1 M of OMG in the method of Toner et al., as Toner et al. disclose that these concentrations of sugar are effective for cell preservation. 'One of ordinary skill in the art would have expected these concentrations of OMG to have been effective for cell preservation.

In view of the foregoing, a holding of obviousness is required.

No claim is allowed.

The Examiner admits that Toner does not teach the use of a non-metabolizable preservation agent. However, Toner also lacks a teaching of using a transporter molecule to load the cell with the cryopreservation agent to a desired concentration sufficient for preserving the cells. Instead, Toner expressly teaches using reversible poration to load cells with otherwise non-permeable sugars. The Examiner is correct that the mammalian cells used in the Toner examples have glucose transporter molecules (the Examiner cites Gould for this purpose) – but the present claims are not claims to a cell, rather these are claims to a method, and the method requires the preservation agent to be loaded to an intracellular concentration sufficient for preservation using the transporter molecules. No reference fills in this missing teaching in the method, and the base reference, Toner, teaches the exact opposite.

Pesquero cannot fill in this missing teaching. The very first sentences of the Pesquero summary provide:

Glucose metabolism has been studied in *Salmo trutta* red blood cells. From non-metabolizable analogue (3-O-methyl glucose and L-glucose) uptake experiments *it is concluded that there is no counterpart to the membrane transport system for glucose found in mammalian red blood cells.*

This reference, cited for the purpose of showing glucose analogue uptake, actually showed an uptake so low that *the authors concluded that there was no transporter molecule* for transporting the agent across the membrane. Indeed, by Applicant's calculation, the intracellular level of 3-O-methyl glucose is on the order of 0.0004M – not an amount sufficient to preserve the cell even if a transport molecule was present.

Even if reference did exist that used transporter molecules to load a non-metabolizable sugar to a predetermined level sufficient to preserve the cells, it could not be combined with Toner at least because (1) it would frustrate the intent and purpose of Toner; and (2) there is no reasonable likelihood of success based on the art.

According to MPEP § 2143.01(V) (at 2100-140), “[i]f [the] proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the propose modification” (citation omitted). Further, in accordance with MPEP § 2143.01(VI) (at 2100-141), “[i]f the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious” (citation omitted). Here, Toner expressly teaches the poration of cell membranes for the purpose of loading otherwise non-permeable sugars. A hypothetical modification to load the cells with permeable sugars through transporter molecules would be the exact opposite of Toner's goal and purpose. Accordingly, no such combination could be made.

In addition, the prior art can be modified or combined to reject claims as *prima facie* obvious only if there is a reasonable expectation of success. *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986); MPEP § 2342.03 (I) at 2100-141.¹ Here, no reference suggests that cells, and especially mammalian cells, can successfully be loaded to sufficient

¹ See, also, *In re Rinehart*, 531 F.2d 1048, 189 USPQ 143 (CCPA 1976) (Claims directed to a method for the commercial scale production of polyesters in the presence of a solvent at superatmospheric pressure were rejected as obvious over a reference which taught the claimed method at atmospheric pressure in view of a reference which taught the claimed process except for the presence of a solvent. The court reversed, finding there was no reasonable expectation that a process combining the prior art steps could be successfully scaled up in view of unchallenged evidence showing that the prior art processes individually could not be commercially scaled up successfully.); and *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 1207-08, 18 USPQ2d 1016, 1022-23 (Fed. Cir.), cert. denied, 502 U.S. 856 (1991) (In the context of a biotechnology case, testimony supported the conclusion that the references did not show that there was a reasonable expectation of success.);

levels with non-metabolizable sugars. Indeed, one would expect that loading cells with intracellular concentration sufficient to change intracellular chemistry as required by the invention would impact the cells being loaded – and this is exactly what the inventors found:

[0064] Viability Assessment after Glucose Loading

[0065] The conventional permeable cryoprotectants such as DMSO and glycerol benefit cells for preservation, but they are known to be toxic to the cells at the same time. *Non-metabolizable glucose compounds are considered to be less toxic because they are metabolically inactive, yet high accumulation of D-glucose may harm glycolysis and glycogen synthesis.* The toxicity of various glucose compounds, D-glucose and DMSO was examined after loading. Cells were incubated with glucose-free DMEM supplemented with 0.2 M 3OMG, with 0.2 M 2DG or 1.4 M DMSO for 60 min at 37.degree. C. After loading, calcein and ethidium homodimer were added to cell suspensions to assess viability. Cells were run through Beckton-Dickinson FACSCalibur flowcytometer to take emission reading at 530 nm and 630 nm of 5000 particles. High green and low red fluorescence (calcein positive) were scored as live, whereas high red and low green (ethidium positive) were scored dead. FIG. 4 showed the percentage of dead cells after incubation with glucose compounds or DMSO. None of the glucose compounds showed toxicity to the cells, yet DMSO showed significant toxicity in all kinds of cells.

Change of Metabolic Activity after Glucose Loading

[0066] The data showed that these non-metabolizable compounds were not toxic as DMSO. However, *it was considered to be possible that high accumulation of glucose compounds change metabolic activity of the cells.* Therefore, metabolic activity after sugar loading was measured with the MTT assay. MTT assay is a colorimetric assay based on the activity of mitochondrial dehydrogenase activity. The MTT assay measures the ability of cells to metabolize 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium 6 bromide (MTT). Cells were seeded on 96-well culture plates with 100 .mu.L defined medium supplemented with various glucose compounds according to the experimental design for 1 hr at 37.degree. C. At the end of the treatment period, 10 .mu.L of MT solution (5 mg/mL) were added and incubated for 2 hrs at 37.degree. C. At this time, 100 .mu.L of detergent solution were added to the wells and after 24 hrs of incubation at 25.degree. C., and the absorption value at 570 nm was measured in a microtiter reader.

[0067] As shown in FIG. 5, metabolic activity was slightly decreased with 3OMG loading in all cells, although viability of cells did not change after glucose loading. This *metabolic down-regulation occurred probably due to glucose starvation. On the other hand, significant loss of metabolic activity was found after 2DG loading in lymphocytes and fibroblasts but not in hepatocytes.* Primary isolated hepatocytes are not down-regulated by 2DG because 2DG is known to exclusively affect proliferating cells (Aft et al (2002) Supra). Down-regulation of metabolic activity is also reported to be protective to the cells during storage. Glucose compounds are thought to protect cells from protein and membrane damage, but metabolic down-regulation could be added value for the preservation. The present results show different effects on metabolism with different compounds. Thus different compounds can be chosen and combined according to the biomaterial being preserved.

In summary, no cited reference teaches the use of transporter molecules to load cells with an intracellular level of sugar sufficient to preserve the cells, and in particular, no reference teaches such loading with non-metabolizable sugars. Even if such a reference did exist, it could not properly be combined with the Toner primary reference as it would (1) frustrate the intent and purpose of the Toner prior art; and (2) there is no basis for a reasonable expectation of success from such a combination given the inherent risks as described in the present application.

Accordingly, as each independent claim includes these features, no claim is rendered obvious by the existence of Toner, Gould, and Pesquero.

CONCLUSION


Applicant expressly requests an interview with the Examiner and submits herewith a formal request for interview.

In the event that a petition for an extension of time is required to be submitted at this time, Applicant hereby petitions under 37 CFR 1.136(a) for an extension of time for as many months as are required to ensure that the above-identified application does not become abandoned.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 141449, under Order No. 22727-138.

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Respectfully submitted,



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